

Accession - Open2Dprot sample accession information program pre-alpha version

Welcome To Accession

http://www.lecb.ncifcrf.gov/Open2Dprot/Accession/ and http://open2dprot.sourceforge.net/Accession

Note: This pre-alpha level software is under construction and will change. Not all functionality described is fully implemented or fully debugged. This documentation will undergo revision as the program develops.

Introduction

Accession is a Open2Dprot project Java program to accession sample information into the system for the Composite Sample Database (CSD). The CSD consists of protein expression per (spot) for N samples may be constructed from N sample spot lists. The Accession program aids in the construction of a database describing the sample information, region of interest and grayscale calibration (if applicable for the samples being used).

Accession is a step [1] module in pipeline analysis for the Open2Dprot project.

You can download the Accession program and install it on your computer. Currently, Accession is hardwired to start with the demo samples and with the -gui switch. However, you can add your own samples to the accession database. This initial default will change as the program stabilizes. The remainder of this home page contains links to some screen shots of the interactive GUI. The Web site contains some initial (rudimentary) documentation.

See the Reference Manual for details. You read about downloading and installing the program on your computer. The source code will be put onto open2dprot.sourceforge.net when it is a bit more stable - currently it undergoing major refactoring. Currently, code is available from the CVS server at Open2Dprot at SourceForge.

Examples - samples of screen shots

To give the flavor of running the Accession program, we provide a few screen shots of the graphical user interfaces and some results. You can these images in the list below or view all of the screen shots in a single Web page.

- Initial user interface Report Window
- Initial command line options tool
- Accession information popup editing Window
- Region Of Interest (ROI) popup editing Window
- Grayscale Calibration popup editing Window

Please contact us with suggestions and comments. If you make interesting changes in the source code, please send us a copy and describe your changes so we can merge them in the released version.

Contact
Us

Accession is a contributed program available at Open2Dprot.sourceforge.net/Accession
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Revised: 10/04/2005

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Accession Program Contributors

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The current Accession program is the result of a collaborative effort with contributions from various groups. The current Java program is partly based based on some of the code refactored from the C/Unix GELLAB-II 2D gel spot pairing program (cmpgl2, 1993 version). That in turn had been refactored from a DEC-10/SAIL language version. The new Java version also includes code from the open source Seg2Dgel, CmpSpots, Flicker, and MAExplorer programs.

Many individuals have been involved over the years in its development both as direct collaborators running 2D gel experiments as well as in offering suggestions for improvement including: Lew Lipkin, Eric Lester, Jim Myrick, Mary Robinson, Carl Merril, Heinz Busse, Peter Sonderegger, Pete Rogan, Ye Chung Wu, Steve Aley, Mike Alley, Rob Ashmore, Kyle Upton, Jeff Orr, Mort Schultz, George Carman, Mark Miller, Arthur Olson, Norman and Leigh Anderson, Sam Hanash, Jian Li, and many others in the electrophoresis community. Published descriptions of the algorithms for the GELLAB-II program on the Open2Dprot Web site and are in the References section of this Web site.

The core group developing the Accession program source currently consists of

- * Peter Lemkin
- * Greg Thornwall
- * Jai Evans

Contact us

Accession is a contributed program available at open2dprot.sourceforge.net/Accession

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Accession Reference Manual

Accession Reference Manual

Accession Reference Manual

The following description will tell you what the options are for the Accession and how to run the program - both interactively and from the command line. If you interact with it through the windowing system, then Figure 1 shows a screen view of the Accession reporting window.

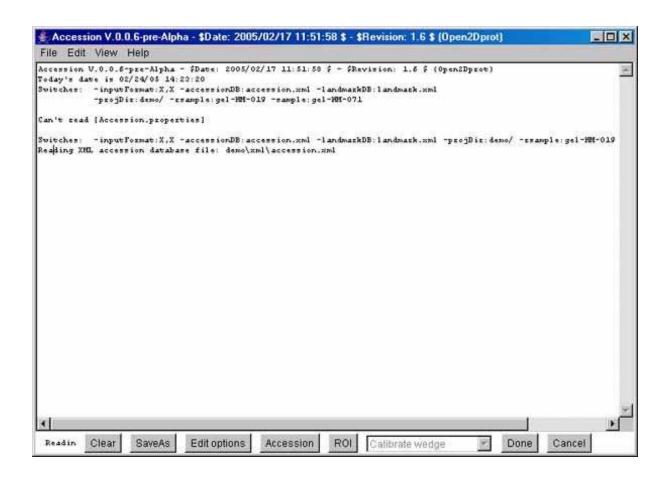


Figure 1. Screen view of the Accession program Report Window interface This screen shot shows the Accession Report Window graphical user interface after starting. There are four pull-down menus at the top of the window: File, Edit, View and Help. There is a row of command buttons and other controls on the bottom of the window. There is a text-report region in the middle of the window that will contain messages, reports and other output from the program. The command buttons Clear and SaveAs respectively clear and save the contents of the report region into a text file. The Edit options button changes the default command line options for the Accession program. The Accession button will popup an accession information editing window for the currently selected sample (File | Open Sample for editing). [See File menu for more information of selecting samples]. The ROI button will popup a Region Of Interest (ROI) editing window for the currently selected sample. The Calibrate pull-down choice will will popup a grayscale calibration editing window for the currently selected sample. There are four calibration choices: Calibrate wedge, Calibrate spot list, Calibrate by file, Calibrate manual data.

1. Introduction

Accession is an open source Java 2D sample accessioning program for entering new samples into the Open2Dprot database. It allows you to associate (currently limited) experiment information with the sample, define regions of interest (for spot segmentation, wedge calibration, etc.), and to calibration image grayscale (if images are used) with particular calibration values (e.g. Optical Density) if a calibration is available. It is part of the Open2Dprot project (http://open2dprot.sourceforge.net/). Accession is similar to the derived the the GELLAB-II 2D gel spot pairing program **getacc** described in the GELLAB-II reference manual. Accession has been generalized to other types of samples besides 2D gels. While the original program was written in C, Accession is written in Java, uses XML input and output files and has an optional graphical user interface. This initial open-source Accession program code could be used as the basis for more advance spot pairing methods. Currently the accession file is accession.xml and is stored in the project "project"/xml/directory.

The program may be run either interactively (-gui) or under an OS shell command line interface to implement batch (-nogui). If the default -gui mode is used, after the spot pairing is finished, the user has the option of interactively examining the paired spot data overlayed on the original sample images. The user may also modify the input switch options and save the new options in a "Accession.properties" file in the current project directory when they exit so that the last used options may be used as the default switch options in subsequent running of Accession.

Project directory structure for Open2Dprot and Accession

All Open2Dprot programs assume a project directory structure. This must exist for the program to proceed. You can either create the structure prior to running any of the programs or you can create it on the fly using the -projDir:user-project-directory. It will lookup and/or create the following sub-directories inside of theuser-project-directory.

The use of these directories is discussed in the rest of this document.

Accession database file

The sample and reference samples to be paired is specified by its image file name using the -sample and -rsample switches with or without the file extension (e.g., -sample:plasma27.tif or -sample:plasma27). The file extension is determined by looking up the image in the ppx/ project subdirectory at run time.

The application looks up the sample in the accession database (in xml/accession.xml or as specified using the -accessionFile switch) and gets additional information about the sample. The Open2Dprot http://open2dprot.sourceforge.net/Accession pipeline module is used for entering samples into the accession database.

[Status: The Open2Dprot Accession module program is not released yet. The accession database could be edited manually as either XML (accession.xml), or tab-delimited text (accession.txt) with Excel.]

There may be images associated with the samples that can be used with the ROI image viewer. This is the case if the spots are to derived from real images (e.g., 2D gels) or virtual images (e.g., from synthesized 2D LC-MS data), etc. These images may be in TIFF (.tif, .tiff), JPEG (.jpg), GIF (.gif), or PPX (.ppx GELLAB-II) format. TIFF images may be 8-bits/pixel through 16-bits/pixel, whereas JPEG, GIF, and PPX are 8-bit images. Gray values in the image files have black as 0. This is mapped after reading to 0 for white and the maximum pixel value for black.

The input sample image files, if any, are kept in the *user-project-directory*/ppx/ sub-directory. This database directory structure is consistent with and is used by the other Open2Dprot analysis pipeline programs.

2. Editing Methods

There are three editing methods: accession information,/A> editing, Region Of Interest editing in the image (if relevant for the types of samples you are using), and grayscale calibration editing.

2.1 Accession information editing popup window

All samples in the accession database must have their associated information defined. Currently, this is defined using the Accession information editing popup window. After editing this data, the accession database should be saved. It is also saved on exiting from the Accession program. [Note: when the new MIAPE standard is integrated with Open2Dprot, the Accession program will be replaced or modified to take this into account].

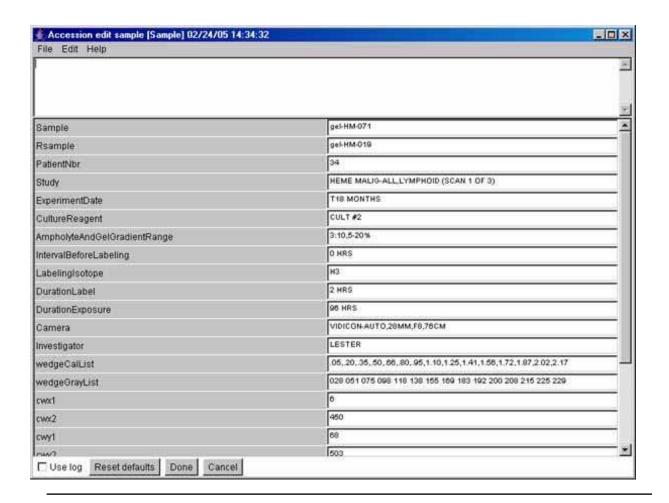


Figure 2.1 Screen view of the Accession information popup editing Window interface. This screen shot shows the Accession Information editor popup Window graphical user interface after starting. You must set the current sample *prior* to starting this editor. There are three pull-down menus at the top of the window: File, Edit, and Help. There is a row of command buttons and other controls on the bottom of the window. There is a scrollable list of Accession DB field names on the left (non-editable) and their corresponding values (editable) on the right. The Checkbox off **Use log** checkbox also copies local text output to the main Accession Report window. The **Reset Defaults** button resets the values to what they were when you first started editing with this popup window. The **Done** button saves any changes and exits this popup information editor.

2.1.1 Pull-down menus in the accession information editing popup window

The menu bar a the top of the Accession Information editing Window contains three menus. The menu notation is specified in Section 5.

- 1. Accession Info File menu to open the options in editing the sample information.
- 2. Accession Info Edit menu to set or clear various editing field options.
- Accession Info Help menu provide documentation on the Accession information editing window.

2.1.1.1 Accession Info File menu

- Use values from template sampleIndicates that the menu has a submenu use the values from the specified template sample for this sample being edited. It does NOT copy the
- Checkbox off **Log text output to Report window** copy all text out to this popup window to the main Accession Report Window as well.
- Save accession DB explicitly save the accession DB if changes were made. Otherwise, it is saved when you press "Done".
- Clear report clear the report text area so that you can start a new report that will be easier to cut and paste or do save to a file
- Save Accession DB save the changes in the accession database file.
- Cancel exit the accession information popup, do not save any changes.
- Exit exit the accession information popup, save any changes when exit.

2.1.1.2 Accession Info Edit menu

These commands are used to change various defaults for the accession information being edited for the current sample.

- Assign current Rsample to this sample assign the current Rsample name to the current sample being edited.
- Clear image computing window ROI entry clear the image computing window ROI entry. This will force you to define the window using the ROI editor popup.
- Clear calibration wedge ROI entry clear the image wedge calibration ROI entry. This will force you to define the window using the ROI editor popup.

2.1.1.3 Accession Info Help menu

These commands are used to request information about Accession information editing.

• **Describe accession fields** - list the accession database fields and the short descriptions of these fields in the top reporting region of the Accession information popup window.

2.2 Region of Interest (ROI) editing

This popup window allows region of interest editing. These are used for calibration in the Accession program and also for defining these regions for their definition for the accession database. If no regions of interest are needed, this step may be skipped.

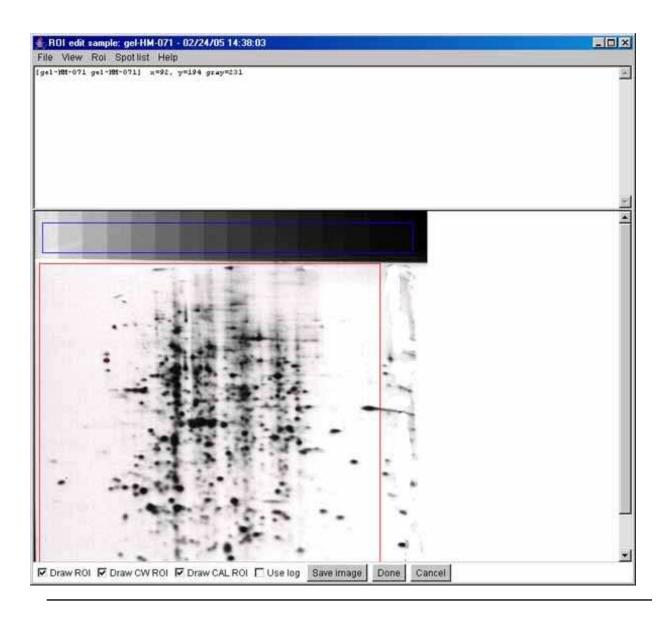


Figure 2.2 Screen view of the Region Of Interest (ROI) popup editing Window interface. This screen shot shows an example of the popup ROI editing Window graphical user interface after starting. ROIs may be used in some circumstances and may not be required in others. There are two ROIs: the image computing window ROI used to specify where the image segmenter is to look for spots to detect; and gray scale wedge calibration ROI used to specify a grayscale calibration wedge (if available in the image). You must set the current sample *prior* to starting this editor. There are five pull-down menus at the top of the window: File, View, Roi, Spot list, and Help. There is a row of command buttons and other controls on the bottom of the window. There is a local ROI reporting window at the top. There is a scrollable image in the center of the ROI editor. The are several overlay option checkboxes: Draw ROI Draw ROI, Draw CW ROI Draw CW ROI, and Draw Calibration ROI Draw CAL ROI. These may also be set from the View menu. The Checkbox on Use log checkbox is used to also copy local output to the main Accession Report window. The Save image button saves the image window with the overlays you have specified into a local GIF image file. The Done button saves any changes and exits this popup ROI editor.

2.2.1 Pull-down menus in the Region Of Interest (ROI) editing popup window

The menu bar a the top of the ROI editing Window contains three menus. The menu notation is specified in Section 5.

- 1. ROI editing File menu to open the options in editing the sample information.
- 2. ROI editing View menu to set or clear various viewing options.
- 3. ROI editing Roi menu to assign, clear, or operate on various ROI options.
- 4. ROI editing Spot list menu to set or clear various spot list manipulation options.
- 5. ROI editing Help menu provide documentation on the ROI editing window.

2.2.1.1 ROI editing File menu

- Log text output to Report window copy all text out to this popup window to the main Accession Report Window as well.
- Save accession DB explicitly save the accession DB if changes were made. Otherwise, it is saved when you press "Done".
- Clear report clear the report text area so that you can start a new report that will be easier to cut and paste or do save to a file
- Save Accession DB save the changes in the accession database file.

• Cancel - exit the ROI popup, do not save any changes.

• Exit - exit the ROI popup, save any changes when exit.

2.2.1.2 ROI editing View menu

These commands are used to change various defaults for the accession ROI being edited for the current sample.

- Checkbox on **Draw trial ROI overlay** draw the trial ROI overlay (cyan color).
- Checkbox on **Draw CW ROI overlay** draw the computing window (CW) ROI overlay (red color). The CW ROI is used to define the image region to segment or find spots.
- Checkbox on **Draw calibration wedge ROI overlay** draw the calibration wedge ROI overlay (blue color). This can be used over a grayscale calibration wedge region in the image.
- Checkbox off **Draw measured spot overlays** draw measured spot overlays (magenta color). This is used if calibration if being performed using a list of spot measurements.
- Checkbox off **Draw measured spot labels** draw measured spot overlay labels (magenta color). This is used if calibration if being performed using a list of spot measurements. The spot labels are "#1", #2", ... "#n".

• **WinDump pixel window size**[IMAGE] - if not "none", i.e. "3x3", "5x5", ... "21x21", show the region around the current pixel.

2.2.1.3 ROI editing Roi menu

These commands are used to request information about Accession ROI editing.

- **Set ROI ULHC at current cursor (C-U)** define upper left hand corner of working trial ROI. Draw a upper corner to indicate this. If both ULHC and LRHC are defined, draw the trail ROI region.
- Set ROI LRHC at current cursor (C-L) define lower right hand corner of working trial ROI. Draw a lower corner to indicate this. If both ULHC and LRHC are defined, draw the trail ROI region.
- Clear working ROI (C-D) clear the working trial ROI.
- Assign ROI to Computing Window (C-C) assign the working trial ROI to the computing window. This is saved in the accession DB for this sample.
- Assign ROI to Calibration Wedge ROI (C-W) assign the working trial ROI to the calibration wedge ROI. This is saved in the accession DB for this sample.
- List current ROI definitions list the trial, CW and wedge ROIs if defined.

2.2.1.4 ROI editing Spot List menu

These commands are used to request information about Accession spot list measurement editing.

- Add measured spot at current cursor (C-M) add spot of current measurement size at the current cursor position to the measurement spot list.
- Remove measured spot at current cursor (C-R) remove selected spot from the measurement spot list.
- **Define common mean background spot at current cursor (C-B)** define a spot to measure mean background to subtract from all spots (optional).
- **List measured spots** List all of the spots and their values in the spot measurement list.
- Clear list of measured spots clear list of measured spots.
- Clear background spot clear background spot (used for background correction).
- Measurement square size[IMAGE] to one of "1x1", "3x3", "5x5", ... "29x29" values. The spot density measurement is the sum of gray values under this square.
- Checkbox on **Recompute spot-list measurements with common size** recompute spot-list with the same common size. This lets you change the spot measurement size for all spots and the corresponding total densities after the spot list was defined.
- Checkbox off **Recompute spot-list measurements with background correction** recompute spot-list with background correction. This lets you add or remove the background correction after the spot list was defined.
- Checkbox off **Sort spot-list measurements** sort the spot-list by density. This lets you keep a sorted spot list even if they were defined out of order.

2.2.1.5 ROI editing Help menu

These commands are used to request information about Accession ROI editing.

• N.A. - (none available yet).

2.3 Grayscale calibration editing

If the samples have associated images, it is sometimes important to calibrate the grayscale of the images with a standard calibration. This is especially important if the stains/dyes or scanner are non-linear to avoid saturation effects in spot quantification. If no grayscale calibration is needed, this step may be skipped.

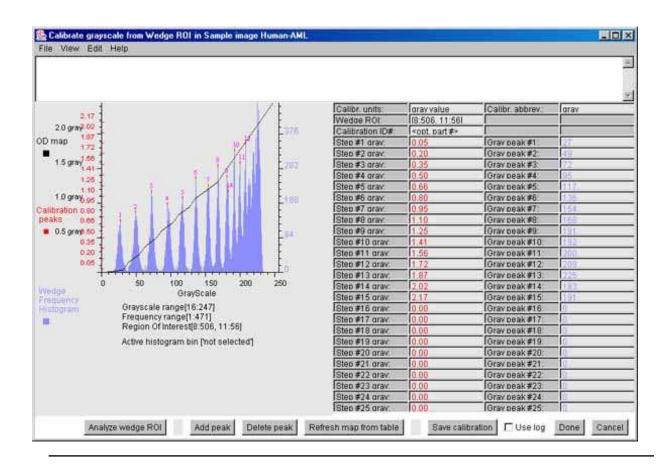


Figure 2.3 Screen view of the Grayscale Calibration popup editing Window interface. This screen shot shows the Grayscale Calibration editor popup Window graphical user interface after starting. You must set the current sample *prior* to starting this editor. There are four pull-down menus at the top of the window: File, View, Edit, and Help. There is a row of command buttons and other controls on the bottom of the window. There is a small text reporting area at the top. There is a grayscale histogram on the left showing various calibration histograms with associated peak values (OD step-wedge histogram shown). There is an editable table on the right where you can have the calibration program estimate the peaks which are then saved in the table. There are other options decribed below. right. The Analyze wedge ROI button computes the grayscale histogram of the wedge in the sample image and updates the table on the right with the histogram peaks that were found. You must have defined the Wedge ROI prior to invoking this calibration popup editor. The Add peak button adds a peak value to the table on the right if you have selected a peak with the mouse in the histogram prior to pressing this button. It will insert a peak if the value you select is between two existing peaks. It redraws the histogram and calibration curve. The **Delete peak** button deletes a peak value to the table on the right if you have selected a peak with the mouse in the histogram prior to pressing this button. It will remove a peak if the value you select is between two existing peaks. It redraws the histogram and calibration curve. The **Refresh map from table** button refreshes the histogram. This is useful if you manually edit the table. The **Save calibration** button saves the calibration in the accession database. The checkbox off Use log checkbox also copies local text output to the main Accession Report window. The Done button saves any changes and exits this popup calibration editor. The **Cancel** button does NOT save any changes and exits this popup calibration editor.

2.3.1 Pull-down menus in the grayscale calibration editing popup window

The menu bar a the top of the Grayscale Calibration editing Window contains three menus. The menu notation is specified in Section 5.

- 1. File menu to open the options in editing the sample calibration
- 2. View menu to set or clear various view overlay options.
- 3. Edit menu to set or clear various editing field options.
- 4. Help menu provide documentation on the Grayscale Calibration editing window.

2.3.1.1 File menu

- Checkbox off Log text output to Report window copy all text out to this popup window to the main Accession Report Window as well.
- Save calibration explicitly save the calibration into the accession DB if changes were made. Otherwise, it is saved when you press "Done".

- Cancel exit the grayscale calibration popup editor, do not save any changes.
- Exit exit the grayscale calibration popup editor, save any changes when exit.

2.3.1.2 Calibration View menu

These commands are used to change various overlay options for the grayscale calibration being edited for the current sample.

• N.A. - Not available yet.

2.3.1.3 Calibration Edit menu

These commands are used to change various defaults for the grayscale calibration being edited for the current sample.

- **Analyze histogram** computes the grayscale histogram of the wedge in the sample image and updates the table on the right with the histogram peaks that were found.
- Add peak (C-A) adds a peak value to the table on the right if you have selected a peak with the mouse in the histogram prior to pressing this button. It will insert a peak if the value you select is between two existing peaks. It redraws the histogram and calibration curve.
- **Delete peak** (**C-D**) deletes a peak value to the table on the right if you have selected a peak with the mouse in the histogram prior to pressing this button. It will remove a peak if the value you select is between two existing peaks. It redraws the histogram and calibration curve.
- **Refresh peak table** refreshes the histogram. This is useful if you manually edit the table.
- Generate linear calibration [***EDIT***]

2.3.1.4 Calibration Help menu

These commands are used to request information about grayscale calibration.

• N.A. - Not available yet.

3. Running Accession and specifying parameter options via the command line

The program may be run either interactively (-gui) with a graphical user interface (GUI) or under an OS shell command to implement batch (-nogui) depending on how it was started. In the former case, after the spot pairing is finished, the user has the option of interactively assigning the accession fields using one or more of the popup windows: Accession, ROI and Calibrate. The user may also modify the input switch options and save the new options in a "Accession.properties" file in the current project directory so that it may be used as the default switch options in subsequent running of the program. [Status: the Accession.properties file is not enabled.] All options including the input reference sample and other sample to be paired are specified via GNU/Unix style switches on the command line (-switch{optional ':parameters'}) and its negation as -noswitch). However, if GUI mode is used, you can interactively specify the switches and their options. It is assigned previously by software that generated the SSF spot lists.

The computing window region of interest

The computing window is a rectangular region or interest in the SSF spot list (real or virtual) image where data is considered to be valid. Spots in this region should be paired. Any spots outside of this region are ignored.

The computing window is defined as [x1:x2, y1:y2]. You can set the computing window using the -cw:x1,x2,y1,y2 command line switch. If you have not defined it or it is not defined in the accession database (if the -accessionFile option is used), it is defined as [0:pixWidth-1 x 0:pixHeight-1] where the virtual image is of size pixWidth x pixHeight.

[STATUS: The computing window for each sample entry is defined in the accession database. The Accession program will allow users to define the computing window.]

Local Folders and files created and used by Accession

When Accession is first started, it will check for the following folders and files in the installation directory and create them if they can not be found.

• Accession.properties - is the default command line switch options to be used (if present). You can edit the default switch options and then save them into the Accession.properties file.

If you specify an image to be semented, it will check whether it is in a ppx/ subdirectory. If not, it will ask you if you want to create a project directory and will then set up the following four directories and copy your image into the ppx/ directory. You can also use the -projDir:user-project-directory switch to specify a (possibly new) project directory.

- batch/ directory holding temporary batch files [NOT USED by Accession]
- cache/ directory holding temporary CSD cache files [NOT USED by Accession]
- ppx/ directory holding your original gel input files
- rdbms/ directory holding CSD database RDBMS files [NOT USED by Accession]
- tmp/ directory holding generated sample image files
- xml/ directory that holds accession DB (created or edited by Accession), landmark DB, SSF spot-list files, and SPF paired-spot list files.

Accession command-line arguments switch usage

The command line arguements usage is:

```
Accession [< optional switches >]
```

The complete list of switches is given later in this manual and as well as some examples of typical sets of switches. The user defined default switches may be specified as a resource string 'Accession.properties' file saved in the project directory. For example:

```
Accession -rsample:gel-HM-19 -sample:gel-HM-071 -project:demo/ -gui
```

Options wizard window for setting the command line switches

If you invoke the **Edit options** button in the Report window (or from the Edit menu), it will popup an options wizard shown in Figure 5 to let you set or change the switch options and then to save these as the new default switch options. The default is saved in the Accession.properties file when you exit program.

Enter sample image and select switch options		
hange the startup parameters, then press 'Set new options' by It that point, you can press 'Pair spots' to pair the Rsample and he new parameters. You may also enter new Rsample and Sa nd/or edit switch options and threshold sliders.	Sample with	
▼ -accessionFile	accession.xml	
-accessionrine;	500000000000	
✓ -backupDatabases	5570	
□ -debugBits	0	
-default	ār sessrī	
▽ -demo	3000	
□ -dtd	(202)	
☑ -inputFormat	×	
▼ -projDir Project directory Browse dir	demol	
-propertiesFile Properties file Browse file	Accession properties	
✓ -rsample Rsample file Browse file	gel-HM-019	
▼-sample Sample file Browse file	gel-HM-071	
-timer	5574	
-update program	program	
□ -usage	· Heti	

Figure 5. Screen view of the popup options wizard window for setting the command line switches, parameter and specifying input samples to be paired. All of the switches are available in the scrollable window. Switches are checked if they are enabled and if the switch requires a value, the current value is shown in the data entry window to its right. On the right there may be several threshold sliders for the upper sizing values for several parameters. In the middle, are several Browse buttons to use for specifying a different samples (**-rsample:** and **-sample:**), and directories. Clicking on any switch will show a short help message associated with that switch at the top of the window. Pressing the **Done** button will pass the new options values back to Accession. Note: aklthough you can use the options wizard to change the Rsample and Sample, it is easier to use the menu comands in the Files menu.

Updating Accession from the Open2Dprot Web server using -update switch

As new versions of Accession are developed and put on the Web server, a more efficient way of updating your version is to use the -update commands. There are four options:

```
-update:program
-update:demo
-update:doc
-update:all

to update the program jar file
to update the demonstration files
documentation files
to update all of the above
```

After updating the program, it should be exited and restarted for the new program to take effect.

Increasing the allowable memory used by Accession

If you are working with very large images that require a lot of memory, you might want to increase the memory available at startup.

If you are using the Windows Accession.exe file or clicking on the Accession.jar file, you can't change the default startup memory.

However, if you are using the Accession.jar in a script using the java interpreter as in the following example which uses the **-Xmx256M** (specifying using 256Mbytes at startup). Change 256 to a larger size if you want to increase startup memory.

```
java -Xmx256M -jar Accession.jar {additional command line args}
```

4. Command and Report Window - the command center

Accession is designed to be used efficiently in a batch mode with minimal command line output. It is also designed to optionally provide a graphical user interface (GUI) which creates a Report Window that captures a report of the spot pairing output as well as additional output directed to it by the user. There are a set of pull-down menus as well as a set of buttons for often used functions.

All logged output is sent to the report window in a scrollable text window that may be saved or used for cut and paste operations. A set of command buttons at the bottom of the window are replicates of commands in the menus, but are easier to access. They include the following functions:

- Status area reports the current status of the spot pairing as it is processing the image.
- Clear clear the report screen
- SaveAs save this report screen in a text file (user is prompted for the file name)
- **Edit options** popup an Options Window to let the user set/clear switches, assign switch parameters (if any), adjust the threshold sizing limits, and specify input samples.
- Accession popup the accession information popup editor
- ROI popup the Region Of Interest (ROI) popup editor
- Calibrate popup the Calibration window to calibrate by one of the choices: "Calibrate wedge", "Calibrate spot list", "Calibrate by file", "Calibrate by manual data".

- **Done** Save accession database changes if required. Then save the Accession.properties file, and exit the Accession program.
- Cancel exit the program. If you need to save changes, ask the user first. If they say no, do not save the changes. Save the Accession properties file, and exit the Accession program.

5. Main pull-down menus in the Accession Graphical User Interface (GUI)

The menu bar a the top of the Report Window contains four menus.

- 1. File menu to open the samples to be paired and other file operations.
- 2. Edit menu to change invoke various editing and other options including command line switches, computing window, and memory size
- 3. View menu to view accession data from the accession database.
- 4. Help menu popup Web browser documentation on the Accession program.

Menu notation

In the following menus, selections that are *sub-menus* are indicated by a 'Indicates that the menu has a submenu'. Selections prefaced with a 'Checkbox on' and indicate 'Checkbox off' indicate that the command is a checkbox that is enabled and disabled respectively. Selections prefaced with a 'Checkbox on' and indicate 'Checkbox off' indicate that the command is a multiple choice "radio button" that is enabled and disabled respectively, and that only one member of the group is allowed to be on at a time. The default values set for an initial database are shown in the menus. Selections that are not currently available will be grayed out in the menus of the running program. The command short-cut notation **C-key** means to hold the Control key and then press the specified *key*.

5.1 File menu

These commands are used to open the samples to be paired and other file operations. The current menus and the menu commands (non-working commands have a '*' prefix) are listed below. You can use either the "Edit options" button to popup the Options Window editor to change the input samples or the (File menu | Open Rsample) and (File menu | Open Sample) commands.

- Create project (directory) create a new project directory and create the new directory and (batch/, cache/, ppx/, rdbms/, tmp/ and xml/) subdirectories. See the -projDir:new project-directory switch that you can use to do this on startup.
- **Set project directory** set the new default project directory and create the new directory and (batch/, cache/, ppx/, rdbms/, tmp/ and xml/) subdirectories if needed. See the -projDir:new_project-directory switch that you can use to do this on startup.

• Open accession file - Open an accession file in the current project directory. Close the old accession file first if it was open.

- **New accession file** Create a new accession database file in the current project directory. Close the old accession file first if it was open.
- Rename of current accession file rename the current accession database. This lets you support multiple accession databases in the same project directory.

• Add sample to the accession DB - add a new sample name to the accession DB. This does not specify the accession info, ROI, or calibrations. You must make this the active sample and then

assign these values using the Edit functions.

- **Set Rsample**[IMAGE] specify the reference sample from samples in the current accession database.
- **Set Sample**[IMAGE] specify the current sample from samples in the current accession database.

• Save accession DB - explicitly save the accession DB if changes were made. Otherwise, it is saved when you press "Done".

- Clear report clear the report text area so that you can start a new report that will be easier to cut and paste or do save to a file
- Save report to a file save the report to a text file

- **Update**[IMAGE] update Accession programs and data from open2dprot.sourceforge.net/Accession server
- Accession all files to get the latest files
- Accession program to get the latest program release (Accession.jar) and other version dependent files from Accession server
- Accession demo files get latest demo project data files
- Accession documentation to get the latest documentation (for stand-alone operation)
- Exit exit the program when in GUI mode.

5.2 Edit menu

These commands are used to change various defaults. These are saved when you save the state and when you exit the program.

- Accession info popup the Accession info editor for the current Sample.
- Regions of interest popup the Regions Of Interest (ROI) info editor for the current Sample.

- Calibrate by step wedge popup the Calibrate by Step Wedge editor to calibrate grayscale by computing the peaks in a calibration wedge scanned with the image for the current Sample.
- Calibrate by spot list popup the Calibrate by spot list editor to calibrate grayscale by a list of measured spots for the current Sample.
- Calibrate by file data popup the Calibrate by file data editor to calibrate grayscale from a file for the current Sample.
- Calibrate by manual entry popup the Calibrate by manual entry of the calibration for the current Sample.
- Read calibration values from file calibrate data from a file.

- Options[IMAGE] change the command line options
- **Open options file** read a new options file (this overides the previous options file which could be Accession.properties).
- Save options file write the current options to the current options file (default Accession.properties)
- SaveAs options file write the current options to a new file you specify
- Edit options popup the an Options wizard window to to edit the switch options and their

parameters, threshold parameter sliders, and input image file

• *Reset default options - reset options to the initial default

5.3 View menu

This menu contains commands to inspect accession database data.

• List accession samples - list the names of all samples in the accession database (in the running program). If you have added samples, this may contain samples not yet in the accession database file.

- Show Rsample accession DB data show accession data for the currently selected Rsample.
- Show Sample accession DB data show accession data for the currently selected Sample.

• Show accession DB in popup browser - show accession database in a popup XML browser.

• Show list of measured spots - show list of measured spots used for calibration.

5.4 Help menu

These commands are used to invoke popup Web browser documentation on Accession. Some of the commands will load local documentation in the the GUI report window.

The documentation is kept on the Internet at http://open2dprot.sourceforge.net/Accession. Normally, these help commands should pop up a Web browser that directly points to the Accession Web page. If your browser is not configured correctly, it may not be able to be launched directly from the Accession program. Instead, just go to the Web site with your Web browser and look up the information there.

- Accession Home the open2dprot.sourceforge.net/Accession home page
- **Reference Manual**[IMAGE] this reference manual
- 1. Introduction
- 2. Editing Methods
- 3. Running Accession
- 4. Command and Report window
- 5. Menus
- 6. Installing Accession
- 7. Command option switches
- 8. Demonstrations
- 9. References
- Sample screen shots examples several screen shots
- Status status of the program bugs and future options
- Revision history show latest Accession release and bugs that were fixed
- Contributors to the spot pairing program sub-project
- PDF files additional documentation including this manual

• Local PDF manual - popup a PDF browser on the local copy of the PDF document that is also available on the Web site

- Open2Dprot home home page for the Open2Dprot project
- License the open source license

• **About** - display information about the program

6. Downloading, installing and running Accession

The installation packages are available for **download** from the SourceForge Files mirror. Look for the most recent release named "Accession-dist-V.XX.XX.zip". These releases include the program (both as Windows .exe file and a .jar file), required jar libraries, demo data, Windows batch and Unix shell scripts. Download the zip file and put the contents where you want to install the program. Note that there is a Accession.exe (for Windows program). You might make a short-cut to this to use in more easily starting the program. Alternatively, you can use the sample .bat and .sh scripts to run the program explicitly via the java interpreter. Note that this method assumes that you have Java installed on your computer and that it is at *least* JDK (Java Development Kit) or JRE (Java Runtime Environment) version 1.5.0. If you don't have this, you can download the latest version free from the java.sun.com Website.

Startup Accession icon

Figure 6. Startup icon for Accession.exe in Windows. Clicking on the icon starts Accession. To start Accession, click on the startup icon shown in Figure 6 below - or you can run the demo-Accession.bat script. For Unix systems including MacOS-X, you can start Accession from the command line by clicking on the Accession.jar file or using the demo-Accession.sh script. You could make short-cuts (Windows) or symbolic-links in Unix to make it easier to start.

6.2 Requirements: minimum hardware and software requirements

A Windows PC, MacIntosh with MacOS-X, a Linux computer or a Sun Solaris computer having a display resolution of at *least* 1024x768. We find that a 1024x768 is adequate, but a 1280x1024 screen size much better since you can see the Popup Report window, Options window, and Accession, ROI or Calibration window at the same time. At least 30 Mb of memory available for the application is required and more is desirable for comparing large images or performing transforms. If there is not enough memory, it will be unable to load the images, the transforms may crash the program or other problems may occur.

An Internet connection is required to download the program from the Open2Dprot Accession Web site. New versions of the program and associated demo data will become available on this Web site and can be uploaded to your computer using the various (**File | Update | ...**) menu commands. If you have obtained the installer software that someone else downloaded and gave to you, then you do not need the Internet connection to install the program. We currently distribute Accession so that it uses up to 256Mb. See discussion on increasing memory by editing the demo- startup scripts.

6.3 Files included in the download

The following files are packaged in the distribution you install. you can periodically a (File | Update from Web server | ... program) menu command to update the files from the open2dprot.sourceforge.net Web server.

- Accession.jar is the Java Archive File for Accession that is executed when you run Accession.
- Accession.exe is the Windows executable file that packages Accession.jar with a java startup. This was packaged using the open-source launch4j (launch4j.sourceforge.net) program.
- Open2Dprot-SSF.dtd is the XML .dtd file used to for the SSF .xml file.

- **Open2Dprot-SPF.dtd** is the XML .dtd file used to generate the SPF .xml file if the -spfFormat:X switch option is used. Note: this is only in generating the SPF file if -dtd switch is set.
- **Open2Dprot-Accession.dtd** is the XML .dtd file that can be used with the accession database file Accession.xml.
- **Open2Dprot-Landmark.dtd** is the XML .dtd file that can be used with the landmark database file Landmark.xml.
- **PDF/fullAccessionDoc.pdf** is a copy of the Accession Web site for use as a reference manual when using the program off-line. It may not be as up to date as the one on the web site but you can do a (File | Update from Web server | Accession documentation) menu command to update it.
- jai_core.jar is the core Java runtime from SUN's Java Advanced Imaging (JAI) at java.sun.com
- jai_codec.jar is the JAI tiff file reader from SUN's Java Advanced Imaging JAI at java.sun.com
- xercesImpl.jar is the Xerces 2 XML SAX/DOM XML library from Apache xml.apache.org
- xml-apis.jar is the Xerces 2 XML SAX/DOM XML library from Apache xml.apache.org
- **license**/ is contains the Mozilla.1.1.html and LEGAL.txt files for Accession and the release-license-jai_files for the Java Advanced Imaging .jar files used by the TIFF file reader.
- **demo/** is a demonstration project directory containing the the following subdirectories:
 - 1. **ppx/** directory holds sample image files (4 images)
 - 2. tmp/ directory holds generated image files
 - 3. **xml**/ directory that the accession DB, landmark DB, input SSF and SPF output files You can do a (File | Update from Web server | Accession demo files) menu command to update it.

7. List of the command line switches

The command line usage is:

```
Accession [< optional switches >]
```

where the order of arguments is not relevant. In the following list, items in **bold** are specific values which must be used (e.g., for -inputFormat: $\{X \mid T\}$, whereas variable values in *italics* indicate that a numeric value for that variable should be used. Some switches have several alternate fixed choices in which case this indicated as a list of bolded items inside of a set of ' $\{...\}$ ' with '|' separating the items. You must pick one of the items and do not include the ' $\{\}$ ' brackets. Also, do NOT include any extra spaces in the arguments of the switch - it will be counted as if it were another switch.

Command line switches

```
-accessionFile:accFile to overide the default accession
     database file. (Default is -noaccessionFile:accession.xml).
-backupDatabases makes backups of the accession and landmark databases "+
      "if they are edited. (Default is -commutativeLMS).
-debugBits:bits,optLandmarkNbr dumps various conditional
     debugging parameters onto the report window. The 'bits' are the
     debug bits specified as either octal or decimal and enable
     particular debugging output if the program was compiled with
     debugging enabled. (Default is -nodebug).
-default sets the default switches to a specific configuration:+
         -nodemo
        -inputFormat:X -projDir:demo/,
        -accessionDB:accession.xml.
     This disables -demo if it was set. (Default -nodefault).
-demo sets the default switches and sample input sample to a specific
     configuration. This may be overriden by turning off the -demo
```

```
switch in the Options Wizard.
-dtd adds the XML DTD file in the output XML file. (Default is -nodtd).
-gui to start the spot pairing with a popup Graphical User Interface
     rather than in batch mode. This captures messages from Accession.
     You can then cut and paste the results or save it to a text
     file. The GUI is also used to change the switch options, re-run
     the spot pairing and view images after each analysis. (Default
     is -noqui).
-inputFormat:<accMode> defines the input formats for
     the accession database file accMode). Where mode is: 'T'
     for tab-delimited (.txt) data, and 'X' for XML (.xml) data.
     (Default is -noinputFormat:X)
-projDir:alternate project directory path to specify the
      project directory to use instead of the default 'demo/' file in
      the installation directory. (Default is -noprojDir).
-propertiesFile:alternate 'Accession.properties' file to specify
      the alternate startup properties file to use instead of the
      default 'Accession.properties' file. (Default is
      -nopropertiesFile).
-rsample: <Rsample file> is the reference sample.
-sample: <Sample file> is the Sample to be paired with the Rsample.
-timer enables a timer to capture processing times for each step.
       (Default is -notimer).
-update:{all | program | demo | doc} specifies that all of the
      Accession files, the program jar files, the documentation files
      or the demonstration files should be updated from the
      Open2Dprot Web server. The program should be exited and
      restarted after updating the program for this to take
      effect. (Default is -noupdate).
```

7.1 Examples of some typical sets of switches

-usage prints all of the switches. (Default is -nousage).

```
The following shows a few examples of useful combinations of command line switches.

Any case-independent switch may be negated by preceding it with a 'no' eg. '-notimer'.

The command line syntax used to invoke it is:

Accession [< opt.-switches >]

The following examples using switches might be useful:

Accession -gui -project:demo/

# Start Accession and load the accession database from the demo project.

Accession -rsample:gel-HM-019 -sample:gel-HM-071 -gui -project:demo/

# Start Accession with two samples already in the accession database
```

7.2 Debug option bits for the -debug switch

The following are the orthogonal octal -debug option code bits. This means you can add them together (in octal) and use that computed octal number (it will also accept decimal). The -debug: bits, landmarkNbr option is meant only for serious programmers reading or modifying the source code.

```
Debugging option bits used with the "-dbug:bits,lmNbr" command line command
```

The following are the orthogonal -debug option code bits in octal. This means you can add them together (in octal) and use that number.

8. Demonstrations

*** BEING REWRITTEN ***

8.1 Examples - samples of screen shots

To give the flavor of running the spot pairing program, we provide a few screen shots of the graphical user interfaces and some images generated by the program.

You can these images in the list below or view all of the screen shots in a single Web page.

- 1. Initial Report Window
- 2. Initial command-line options tool
- 3. Popup Accession info editor
- 4. Popup Region Of Interest (ROI) editor
- 5. Popup Calibration editor

8.2 Example - output of the Report Window

The following Report Window output was generate for the images in the above example.

8.3 Examples - using command line processing for batch

It is possible to run the Accession from the command line in your operating system. We give two examples doing this. The first example shows a script for the Microsoft Windows batch (.bat) file for processing 4 images demo-Accession.bat file (available on the Files Mirror. The second example shows the same commands in a shell script for a Unix operating system (Linux, MacOS, Solaris, etc.) is available at from the Files mirror at demo-Accession.sh.

The data for these scripts is in the **demo**/ directory available with the installation which also includes the scripts. Alternatively, you can download the demo data from the Files Mirror as Demo.Zip.

The installation packages are available from the Files mirror under the Accession releases.

8.3.1 Examples - batch processing under Microsoft Windows

```
REM File: demo-Accession.bat - accession a sample gel
REM
REM This example assumes that all .jar files listed below and demo/ directory are
REM in the current directory. Modify for other situations.
REM
REM The JDK should be installed and version 1.4 or later is required.
REM You can download the latest JDK from http://java.sun.com/
```

```
REM The files needed are listed below:
REM JAR files required and mentioned in manifest:
       xml-apis.jar xercesImpl.jar jai_codec.jar jai_core.jar 02Plib.jar
REM demo Files:
       demo/ppx/gel-HM-019
REM Accession database file is in:
REM
        demo/xml/accession.xml
REM Generated Sample Spot-list Files (SSF) are saved in:
REM
        demo/xml/
REM Generated images are saved in:
REM
        demo/t.mp/
REM
REM P. Lemkin $Date$
echo "demo-Accession.bat"
pwd
date /T
java -Xmx256M -jar Accession.jar -demo -gui -project:demo\
echo "-- Finished accessioning the samples ---"
date /T
```

8.3.2 Examples - batch processing under Unix (or MacOS-X)

Because java is relatively operating system independent, the same java command lines are used with the "\" changed to "/", "REM" changed to "#", and "DATE/T" to "date" from Windows to Unix script and file path conventions.

```
#!/bin/sh
# File: demo-Accession.sh - accession a sample gel
# This example assumes that all .jar files listed below and demo/ directory are
# in the current directory. Modify for other situations.
# The JDK should be installed and version 1.4 or later is required.
# You can download the latest JDK from http://java.sun.com/
# The files needed are listed below:
# JAR files required and mentioned in manifest:
     xml-apis.jar xercesImpl.jar jai_codec.jar jai_core.jar 02Plib.jar
# demo Files:
      demo/ppx/gel-HM-019
# Accession database file is in:
      demo/xml/accession.xml
# Generated Sample Spot-list Files (SSF) are saved in:
     demo/xml/
# Generated images are saved in:
      demo/tmp/
# P. Lemkin $Date$
echo "demo-Accession.sh"
pwd
date
java -Xmx256M -jar Accession.jar -demo -gui -project:demo/
echo "-- Finished accessioning the samples ---"
date
```

9. Accession References

These papers (a subset of the GELLAB-II papers), reference the GELLAB-II spot pairing program. The Open2Dprot Java-language Accession program was other Open2Dprot programs and concepts were used from the old GELLAB-II C-language program as well as from code from the MAExplorer and Flicker projects. This program will be replaced with a full XML editor that will make use of the new MIAPE standard. New Java code was added as well. Although Accession has been enhanced in many ways, the basic methods are similar so these papers may be useful for more details on the algorithm.

- 1. Lipkin, L.E., Lemkin, P.F. (1980) Database techniques for multiple PAGE (2D gel) analysis. *Clinical Chemistry* **26**, 1403-1413.
- 2. Lemkin, P., Lipkin, L. (1981) GELLAB: A computer system for 2D gel electrophoresis analysis. I. Segmentation and preliminaries. *Computers in Biomedical Research* **14**, 272-297.
- 3. Lemkin, P., Lipkin, L. (1981) GELLAB: A computer system for 2D gel electrophoresis analysis. II. Pairing Spots. *Computers in Biomedical Research* **14**, 355-380.
- 4. Lester, E.P., Lemkin, P.F., Lipkin, L.E. (1981) New Dimensions in Protein Analysis 2D Gels Coming of Age Through Image Processing, Invited paper, *Analytical Chemistry* **53**, 390A-397A.
- 5. Lemkin, P.F., Lipkin, L.E. (1983) Database Techniques for 2D Electrophoretic Gel Analysis, in Computing in Biological Science, Elsevier/North-Holland, M. Geisow, A. Barrett (eds), 181-226.
- 6. Lemkin, P.F., Lipkin, L.E., Lester, E.P. (1982) Extensions to the GELLAB 2D Electrophoresis Gel Analysis System. Paper given at "Clinical Applications of 2D Electrophoresis", Mayo Clinic, Nov. 15-18, 1981. *Clinical Chemistry* 840-849.
- 7. Lemkin, P.F., Lester, E.P. (1989) Database and Search Techniques for 2D Gel Protein Data: A Comparison of Paradigms For Exploratory Data Analysis and Prospects for Biological Modeling, *Electrophoresis* **10**(2): 122-140.
- 8. Lemkin, P.F. (1989) GELLAB-II, A workstation based 2D electrophoresis gel analysis system, in proceedings of <u>Two-Dimensional Electrophoresis</u> T. Endler, S.Hanash (Eds), Vienna Austria, Nov 8-11, 1988, VCH Press, W.Germany. pp 53-57.
- 9. Lemkin, P.F., Rogan, P. (1991) Automatic Detection of noisy spots in two-dimensional Southern Blots, *Applied and Theoretical Electrophoresis* **2**, 141-149.
- 10. Myrick, J.E., Lemkin, P.F., Robinson, M.K., Upton, K.M. (1993) Comparison of the BioImage Visage(TM) 2,000 and the GELLAB-II two-dimensional electrophoretic analysis systems. *Applied and Theoretical Electrophoresis* **3**, 335-346.

Contact us

Accession is a contributed program available at open2dprot.sourceforge.net/Accession Powered by SourceForge Logo

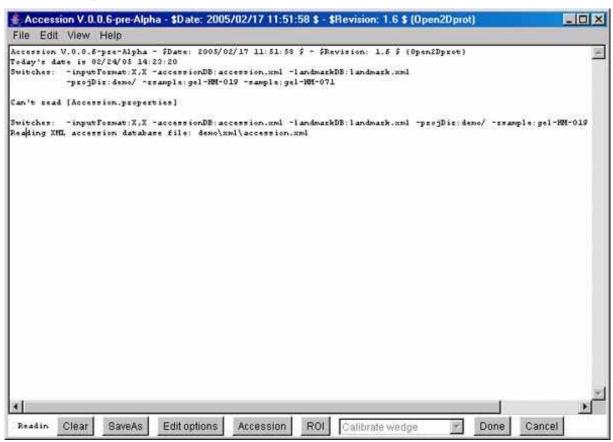
Revised: 05/31/2006

Sample Screen Shots for Accession program - pre-alpha version

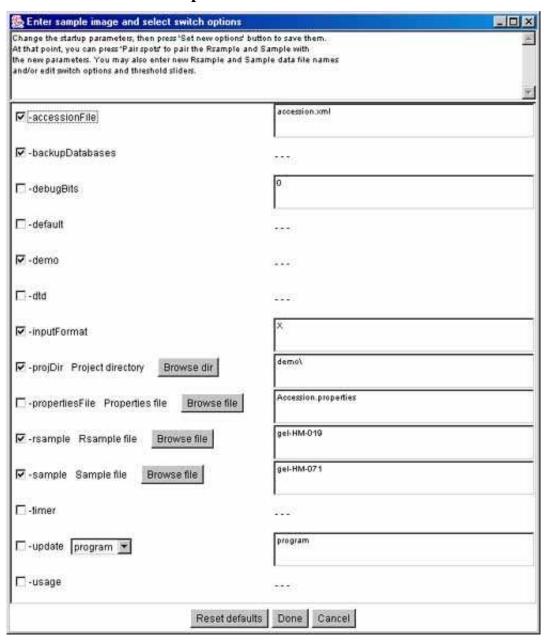
To give the flavor of running the spot pairing program, we provide a few screen shots of the graphical user interface generated by the program.

- 1. Initial user interface window
- 2. Initial command line options tool
- 3. Using Accession data popup editor
- 4. Using image Region Of Interest (ROI) popup editor
- 5. Using grayscale calibration popup editor

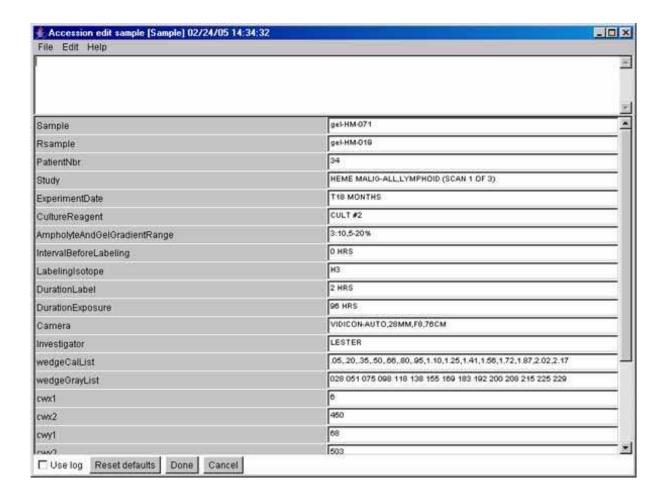
1. Initial Report Window



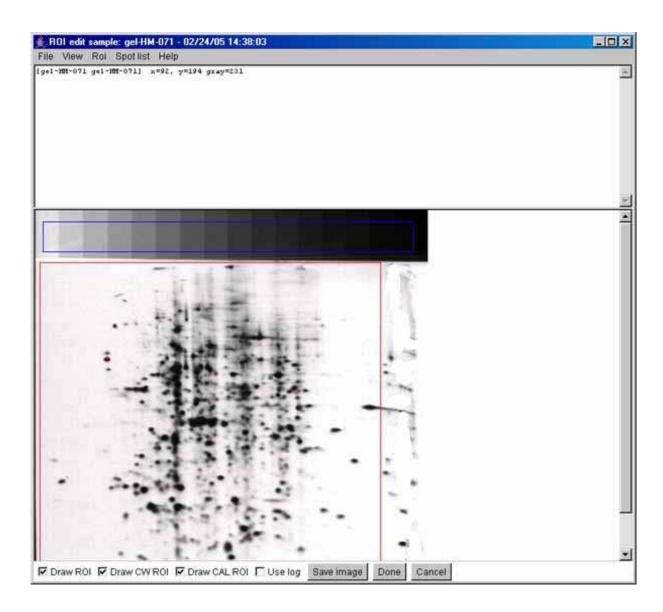
2. Initial command line options tool



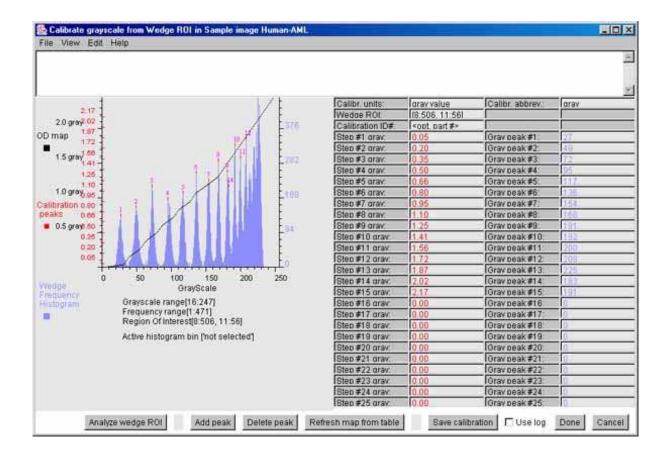
3. Using Accession data popup editor



4. Using image Region Of Interest (ROI) popup editor



5. Using grayscale calibration popup editor



Contact

Accession is a contributed program available at open2dprot.sourceforge.net/Accession

Revised: 10/04/2005

Accession Jar Version

Accession Jar Version

There are two versions of the Accession program available on ${\tt Open2Dprot}$ at the server

http://open2dprot.sourceforge.net/Accession

After you have downloaded and installed the program, you can update it quickly by having Accession copy the Accession .jar file from the Web server using the (File | Update from Web server | Accession program) menu command. After you do the update, you need to restart the program to use the new version.

Until futher notice, Accession is to be considered beta-level code until it is officially released. This means that there may be some functionality not fully implemented, that works incorrectly, or that has changed. New commands and functionality are in the process of being added. Please report problems and suggestions to us.

Accession version 0.16

Accession - Get Acrobat (PDF) documents

There are a number of Adobe Acrobat PDF formated documents and slide shows available for the Accession project.

If you do not have Adobe Acrobat, you can download it for free from Adobe. [IMAGE] The following lists some of the PDF documents you may download. As otheres are created, they will be added to this list.

- 1. Accession Overview slide show for use as a printable document.
- 2. Accession Overview slide show for use as a printable document (2 panels/page).
- 3. Accession Overview slide show for use as a printable document (6 panels/page).
- 4. This Accession Web site as a PDF document for use as a printable document

Contact	Accession is a contributed program available at	Revised:
	1 6	10/04/2005
us	open2dprot.sourceforge.net/Accession	004

Accession Revision History

Accession Revision History

This describes the revision history of the Accession program of released or soon to be released versions. Generally, only the most recent versions are kept on the Web site (see Version). Documentation for unresolved bugs and requested functionality is found in the status Web page.

- **V.0.16 05-31-2006**: Get (File | Updates) from main server jar/, demo/, dtd/, scripts/ etc. directories.
- V.0.15 12-21-2005: Read TIFF images before GIF and JPEG.
- V.0.14 11-07-2005: Changed installer paradigm to use launch4j. Also, new manifest for
 Accession.jar file that now includes the Class-Path so the demo .bat and .sh scripts were
 simplified in this release. The (Edit menu | Resize memory limits) command was depricated see Increasing or decreasing the allowable memory used by Accession in the Reference manual.
- V.0.10 05-11-2005: Rebuilt after refactored O2Plibrary. Now the Accession text editor has
 required fields in red and dynamic (optional) fields in blue. The future MIAPE fields will be
 dynamic.
- V.0.10 04-18-2005: Cleanup and added addition File menu commands.
- V.0.0.7 03-21-2005: Fixed bug in -usage and -version switches.

- V.0.0.6 02-25-2005: Docmentation has been updated.
- V.0.0.6 02-17-2005: Initial Web site version. This version is buggy. The calibration options do not work correctly and is disabled for now. However, it does allow adding a sample and defining image ROIs.

Contact Accession is a contributed program available at Revised: us y open2dprot.sourceforge.net/Accession 05-31-2006

Accession Program Status

Accession Program Status

The following are known bugs, suggested features, functions that are being debugged or being developed. Documentation for resolved bugs is found in the revision history Web page.

- Revision history
- Known bugs
- Suggested Features/FAQ

Known Bugs Known bugs

The neumonic *GGGR* is shorthand for "Grep, Get Gestault and Refactor".

O V.0.0.6 02-17-2005: Initial version.

Suggested Features suggested features

O V.0.0.6 02-17-2005: [TODO]

Contact Accession is a contributed program available at Revised: us open2dprot.sourceforge.net/Landmark 10/04/2005